Virus-based technologies for investigating function and pathology of the nervous system

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Virus-based technologies for investigating function and pathology of the nervous system

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Tools to manipulate gene expression in defined populations of neurons are crucial for progress in understanding the brain and for treatment of nervous system disorders. An increasingly popular approach for experimental and therapeutic manipulation of neuronal gene expression is to transduce neurons using viral vectors. The ENINET (www.eni-net.org) sponsored workshop "Virus-based technologies for investigating function and pathology of the nervous system" took place on October 1st–2nd, 2009, at the European Brain Research Institute (ENI-Rome institution) in Rome, Italy. This workshop addressed the basic biology of viruses, the strengths and weaknesses of different viral systems for experimental and therapeutic applications, new developments in virus-based technologies and their application in investigating nervous system function.

The workshop began with an in depth introduction to two of the most frequently used viral vectors. Isabelle Barde (EPFL) provided a comprehensive overview of the development of lentiviruses as research tools. Lentiviruses, such as human and feline immunodeficiency viruses, have been turned into flexible and safe tools for introducing genetic manipulations into various types of cells including neurons. Applications now extend from routine use of lentivirus vectors to transduce cultured neurons, to *in vivo* expression of transgenes and development of transgenic animals. The next speaker, Nicholas Mazarakis (Imperial College), reviewed the use of both lentivirus and adeno-associated virus (AAV) vectors for gene therapy. After an interesting introduction to the engineering of these viruses for gene transfer in humans, he demonstrated their potential use for several neurological disorders including Parkinson's disease, for which he provided very exciting proof of principle data for reversal of Parkinsonian symptoms. Further examples of the potential clinical importance of AAV vectors were provided in a later talk by Jean-Michel Heard (Institut Pasteur), who gave a detailed account of the development of AAV vectors for human gene therapy to treat lysosomal storage disease. This important example illustrates the progression from recombinant vector conception to ongoing human clinical trials.

Several speakers highlighted the diverse applications of lentivirus vectors. Melanie White (Edinburgh University) described an example of the use of a lentiviral vector-encoded silencing RNA as a potential therapeutic tool to treat prion diseases. Oliver Schlüter (ENI-Göttingen) described the use of a lentivirus vector system to examine the role of the post-synaptic protein PSD95 in synaptic transmission. He explained how he developed a powerful molecular replacement strategy using a combination of lentivirus-encoded silencing RNAs to knockdown native PSD95 and mutant PSD95 transgene constructs to rescue function. Angel Barco (ENI-Alicante) described the development of an exciting novel gain-of-function approach using lentiviruses to unveil the transcriptional programs of specific transcription factors.

Sarah Salinas (IMG-Montpellier) discussed the utility of adenovirus-derived canine serotype 2 (CAV-2) vectors. Development of CAV-2 vectors has overcome the potential toxicity associated with earlier adenovirus vectors, providing an efficacious vector that can carry larger payloads than lentivirus or AAV. As well as reviewing the biology of CAV-2 and its modifications for use in research, this talk highlighted several neurobiological studies that successfully used CAV-2 for gene transfer. Another exciting use of adenoviral systems was introduced by Anthony Pickering (Bristol University), who described the manipulation of the function of noradrenergic neurons involved in pain control. By transducing noradrenergic neurons in the pons with a virus expressing a potassium channel, he was able to produce modality specific hyperalgesia. He went on to suggest how these results will form a basis for future approaches to induce analgesia by activating pontine neurons.

While AAV, adenovirus and lentivirus are perhaps the most frequently used vectors for neuroscience applications, other viruses with different biological properties have proven useful. Nigel Fraser (University of Pennsylvania) provided an excellent overview of the development of the herpes simplex virus (HSV) as a powerful tool for research. While development of this virus as an experimental tool is at an early stage, its relatively large genome (152 kb) gives the potential to carry large payloads and Dr. Fraser provided good examples for its potential use to treat mucopolysaccharide disease and cancer, as well as for the tracing of neural connectivity. Different advantages can be obtained from use of alphaviral vectors, such as Semliki Forest and Sindbis viruses. These were highlighted by Markus Ehrengruber (KSHP), who provided a comprehensive overview of the use of alphaviral vectors, as well as the measles virus, in neurobiology. He illustrated a good example of the use of the Semliki Forest virus expression vector to study the function of the synaptic protein Homer. Particular strengths of the alphaviral vectors are the rapid and high level of transgene expression that can be obtained, although potential toxicity is a drawback for long-term expression studies.

The final session of the meeting showcased examples of viruses used *in vivo* to address basic neurobiological questions. Peer Wulff (ENI-Aberdeen) described a powerful approach to specifically target expression of a toxin in interneurons in the brain by combined use of a mouse line that expresses Cre recombinase specifically in interneurons, with an AAV-loxP-toxin recombinant virus. Using this system, he specifically blocked release of neurotransmitters from interneurons in the hippocampus. Hélène Marie (ENI-Rome) then illustrated the successful use of the recombinant Sindbis virus system in identifying the effects of a constitutive mutant form of the transcription factor CREB on synaptic transmission and memory formation by combining its use for *in vivo* expression with electrophysiological and behavioral analyses. Next, Andreas

Frick (ENI-Bordeaux) provided an introduction to the use of rabies viruses in neuroscience, focusing on its well-known characteristic of retrograde transport. He summarized his work using this virus to label the mono-synaptic input map of neurons in the cortex and retina. Michael Häusser (ENI-London) nicely concluded this final session by showing us how his group combines *in vivo* shadow patching, *in vivo* single cell electroporation and *in vivo* rabies infection of cortical neurons to elucidate the wiring maps of individual neurons.

In conclusion, the workshop demonstrated versatile applications of various viral vectors for basic neuroscience research and for human gene therapy. Some of these vectors have been the focus of considerable recent developments to make them invaluable research tools. However, a recurring theme was that work remains to be done to optimize even the most advanced vectors for *in vivo* delivery and target specificity; while other relatively uncharacterized viruses may provide novel and potentially useful properties that would make them attractive as alternative experimental and therapeutic tools.

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